Dkt 7211.01

NON-PROVISIONAL

PATENT APPLICATION

Entitled

NanoScale Molecular Arrayer

of

Eric Henderson
2807 Torrey Pines Road
Ames, IA 50014

and

Curtis Mosher
601 Garden Road
Ames, IA 50010

I hereby certify that this paper of fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 to: Box Patent Application, Commissioner for Patents, Washington, D.C. 20231 on this 14 day of August, 2001.

Express Mail No.: EL832025601US

Name: ______

TITLE: NANOSCALE MOLECULAR ARRAYER

PRIORITY

This application claims benefit from prior Provisional Application Serial No. 60/225,434, filed August 15, 2000.

FIELD

This invention relates to the generation of solid state molecular arrays. More specifically, this invention relates to a dedicated apparatus for the creation of molecular arrays in a high throughput format with domain sizes as small or smaller than 1 micron in size.

BACKGROUND

Measuring the binding affinity between materials, molecules, and cells is key to a broad spectrum of industries, including material development, semiconductor production, bioanalytical assays, biomedical diagnostics, and drug discovery. With the emergence of solid state array-based bioanalytical and genetic diagnostic instruments and related equipment, new methods for cost effective screening of a large number of reactions in a miniaturized solid state form have become increasingly desirable. The favored approach to date is to monitor changes in optical properties, usually fluorescence, when a known, fluorescently labeled molecule interacts with a known molecular species at a specific address in a molecular array. These apparatuses and methods, however, often impose stereochemical constraints by the addition of reporter systems to the molecules used to interrogate the molecular array. Thus, label free, direct interrogation of molecular binding events using a micromechanical reporter is of obvious utility. More sophisticated and robust instrumentation for the creation of these molecular arrays is therefore desirable.

One method for the direct detection of molecular interaction events is the scanning probe microscope. One type of scanning probe microscope is the atomic force microscope ("AFM"). In the AFM, a sharp tip is situated at the end of a flexible cantilever and scanned over a sample surface. While scanning, the cantilever is deflected by the net sum of the attractive and repulsive forces between the tip and sample. If the spring constant of the cantilever is known, the net interaction force can be accurately determined from the deflection of the cantilever. The deflection of the cantilever is usually measured by the reflection of a focused laser beam from

the back of the cantilever onto a split photodiode, constituting an "optical lever" or "beam deflection" mechanism. Other methods for the detection of cantilever deflection include interferometry and piezoelectric strain gauges.

The first AFMs recorded only the vertical displacements of the cantilever. More recent methods involve resonating the tip and allowing only transient contact, or in some cases no contact at all, between it and the sample. Plots of tip displacement or resonance changes as it traverses a sample surface are used to generate topographic images. Such images have revealed the three dimensional structure of a wide variety of sample types including material, chemical, and biological specimens. Some examples of the latter include DNA, proteins, chromatin, chromosomes, ion channels, and even living cells.

In addition to its imaging capabilities, the AFM can make extremely fine force measurements. The AFM can directly sense and measure forces in the microNewton (10^{-6}) to picoNewton (10^{-12}) range. Thus, the AFM can measure forces between molecular pairs, and even within single molecules. Moreover, the AFM can measure a wide variety of other forces and phenomena, such as magnetic fields, thermal gradients and viscoelasticity. This ability can be exploited to map force fields on a sample surface, and reveal with high resolution the location and magnitude of these fields, as in, for example, localizing complexes of interest located on a specific surface. To make molecular force measurements, the AFM probe may be functionalized with a molecule of interest.

Construction of molecular arrays on a solid support for use in an AFM is typically carried out by processes that can be divided into two general classes: *in situ* and *ex situ*, the latter including a mechanical deposition step to actually place the sample on the deposition surface. *In situ* synthesis methods and apparatuses may involve photochemical synthesis of nucleic acid or short peptides to define the spatial addresses on a silicon or a glass surface. These methods maybe limited by the wavelength of light used for masking and the synthetic procedure. Furthermore, this procedure may also be limited by cost. A need therefore exists for a dedicated apparatus for the creation of molecular arrays that may create the array in a quick and efficient manner.

An example of an *ex situ* method followed by the mechanical deposition on the surface may be illustrated by the "dip pen" method. The sample material is prepared in advance and then the dip pen is used to place the sample on the deposition surface. It has been shown that a

dip-pen method may be used to draw a submicron molecular line or spot using an alkanethiolate monolayer utilizing a standard AFM to control the dip pen. Other prior art instruments may utilize a pin tool which is dipped in a solution containing the sample material. The pin tool then has a drop of solution on it, which is then placed on the deposition surface. This method, however, does not allow the creation of extremely small deposition domains. Up until this time, AFMs have been utilized for drawing sub-micron molecular lines or creating the molecular spots. AFMs, however, are not optimal for creating arrays because they lack features, such as a sub-micron precision sample stage under computer control, precise optical access for sample registration, and unencumbered access to the software code used to control the tip motion. Furthermore, commercial AFM configurations are not amenable to the rapid deposition of large numbers of different molecular species. Finally, AFMs are designed for multiple tasks, not as a dedicated sample deposition instrument, and are therefore more expensive than is required for a dedicated arrayer. Still other features may also be desirable in a dedicated deposition instrument and not included with an AFM. A need therefore exists for an instrument that is dedicated to the creation of arrays comprised of deposition domains.

A need exists for a commercially practical deposition instrument that can be utilized to create a molecular deposition array that includes sub-micron deposition domains. This instrument may incorporate precise optical features for sample registration and may be controlled utilizing a computer control so that user defined array patterns and sizes may be created. It may be particularly advantageous if this instrument can operate autonomously in a high throughput format.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a block figure representing the various components of the one embodiment of the present invention.

Figure 2 is a front view of the instrument of one embodiment of the present invention.

Figure 3a is a perspective view of the X, Y controller of one embodiment of the present invention.

Figure 3b is a perspective view of the X, Y translation stage of one embodiment of the present invention.

Figure 4 is a perspective view of the deposition probe of one embodiment of the present invention.

Figure 5 is a block diagram showing the components of the humidity controller of the present invention.

SUMMARY

An apparatus for creating molecular arrays comprising a base, a Z controller operably connected to the base wherein the Z controller is selectively positionable along a Z axis, a deposition probe removably and operably connected to the Z controller so that the deposition probe is selectively positionable along the Z axis by the Z controller, an X, Y controller operably connected to the base wherein the X, Y controller is selectively positionable along an X axis and a Y axis, the X, Y controller further comprising a deposition substrate operably attached thereto and wherein the movement of the X, Y controller moves the deposition substrate between a first position and a second position, the second position being operably positioned relative to the deposition probe, and an X, Y translation stage operably connected to the base wherein the X, Y translation stage is selectively positionable along an X axis and a Y axis, the X, Y translation stage further comprising a loading substrate operably attached thereto and wherein the movement of the X, Y translation stage moves the loading substrate between a first position and a second position, the second position being operably located relative to the deposition probe and the first position being in a position accessible by the user.

A method for creating a deposition domain comprising (a) obtaining a loading substrate, the loading substrate further including a deposition material, (b) loading the deposition material onto a deposition probe, and (c) creating a deposition domain on a deposition substrate by transferring a desired amount of the deposition material from the deposition probe to the deposition substrate.

An apparatus for creating an array comprising, a Z controller, a deposition probe operably attached to the Z controller, the deposition probe further comprising a tip, an X, Y controller operably attached to the Z controller, the X, Y controller selectively movable between a first position and a second position, and a deposition substrate operably affixed to the X, Y

controller wherein when the X, Y controller moves the deposition substrate to the second position the deposition substrate is operably positioned relative to the deposition probe.

The present invention is a dedicated instrument for the creation of molecular arrays comprising deposition domains as small or smaller than 1 micron. Utilizing the present invention arrayer may limit the use of expensive reagents and test materials and may further help to conserve space in large scale combinatorial chemistry labs. Finally, the present invention may permit the testing of a large number of samples in a high throughput format because of the ease of making custom designed arrays with a variety of deposition materials placed thereon.

The present invention apparatus utilizes a deposition technique in which the sample is transiently hydrated to form a capillary bridge. The capillary bridge may transport the deposition material from the loading substrate, to the deposition probe, and from the deposition probe to the deposition substrate, to create a deposition domain. One or more deposition domains make up the array. The capillary bridge deposition technique utilized by the present invention apparatus is further described herein, and is also described in detail in co-pending U.S. Application 09/574,519, which is herein incorporated by reference for all that it teaches.

DETAILED DESCRIPTION

The specification describes an arrayer 10 that creates arrays comprised of deposition domains in a high throughput format. In one embodiment the arrayer is automatically controlled, bypassing the need for a user to constantly monitor the formation of the array. A general description of the components of the arrayer 10 will be undertaken followed by a more specific description of each component.

As illustrated in Figures 1 and 2, one embodiment of the present invention arrayer 10 may be comprised of a deposition probe 12, an X, Y, controller 14, a Z controller 16, an X, Y translation stage 18, a humidity controller 20, a control computer 22, and a base 24. The deposition probe 12 may be operably connected to the Z controller 16 which in turn may be affixed to the base 24. The X, Y controller 14 may also be affixed to the base 24 on a first side, of the Z controller 16. The X, Y translation stage 18 may further be affixed to the base 24 on a second side of the Z controller 16. The humidity controller 20 and the control computer 22 may be operably positioned relative to the deposition probe 12, the X, Y controller 14, and the X, Y translation stage 18 so that the humidity controller 20 may properly perform its respective

function, i.e., controlling the humidity. The computer 22 controls the function of the various components of the present invention arrayer 10. As may be appreciated, a number of formations and designs imagined by those skilled in the art may be utilized to attach the X, Y controller 14, the Z controller 16, the X, Y translation stage 18, etc. to the base 24. Different orientations of the components does not alter the scope of the present invention. Furthermore, these components may be attached in a number of different ways, including bolting, welding, snapping, etc.

As illustrated in Figure 3a, the X, Y controller 14 further includes a deposition substrate 25 movably and removably affixed thereto. The deposition substrate 25 is the surface upon which the present invention deposits the material. The substrate is moved by the X, Y controller 14 into a position underneath the Z controller 16 so that that deposition probe 12 can be lowered and the deposition material deposited. The substrate 25 may be affixed to the X, Y controller 14 utilizing snaps, clips, raised contours, or by other methods known to those skilled in the art. The details of how the arrayer 10 deposits the material is better understood after an explanation of each of the portions of the present embodiment. In still further embodiments, one controller may control the movement of the deposition probe 12 in the X, Y, and Z directions.

The deposition substrate 25 utilized in the present invention apparatus may be formed of a variety of materials depending on the nature of the deposited material. A further description of such deposition substrates 25 can be found in U.S. Application 09/574,519, but may be altered or changed without changing the nature or scope of the present invention arraying apparatus.

As is further illustrated in Figure 3b, the X, Y translation stage 18 may further include a loading substrate 27. The loading substrate 27 may be the surface on which the deposition material resides before it is loaded onto the deposition probe 12, and then onto the deposition substrate 25, of the arrayer 10. The deposition material may be placed on the loading substrate 27 by methods known to those reasonably skilled in the art, such as by mechanical deposition, *in situ* photochemical synthesis, "ink jet" printing, and electronically driven deposition, without changing the nature and scope of the present invention.

In one embodiment, as illustrated in Figure 2, the arrayer 10 may further comprise a force feedback monitor 50 and an optical microscope 52. The force feedback monitor 50 may be operably connected to the deposition probe 12, the Z controller 16, and the control computer 22. The force feedback monitor 50 may assist the present invention in controlling the height of the

deposition probe 12 relative to the deposition substrate 25 and the loading substrate 27. The optical microscope 52 may be operably attached at a position below the base 24 in such a position to aid the user in observing the action of the arrayer 10.

Each of these separate components of the present invention apparatus will now be further described herein.

Base 24

With reference to Figure 2, the base 24 of the present invention will be herein described. The base 24 of the present embodiment is physically stable and provides various places where the separate portions of the present invention may be mounted. The base 24 of the present embodiment may utilize a 12 x 24 inch optical plate supported on steel posts 26. The optical plate is a standard platform for building various types of instrumentation.

One commercially available optical plate 24 that may be well suited for use in the present invention arrayer 12 may be available from Newport Corp., P.O. Box 19607, Irvine CA 92623-9607 as product number SA12. The plate may have ¼ inch holes drilled on one inch centers. Steel posts 26 well suited for the present invention may also be commercially available from the same manufacturer as product number SP12.

In alternative embodiments, the optical plate may be placed on top of an optical table. The optical table can be floated on nitrogen pistons to optimize the elimination of vibrations, though in the present embodiment it is not necessary to go to such extremes to create arrays with the present invention.

Controller 14

With reference to Figures 2 and 3a, the X, Y controller 14 of the present invention will be herein described. As illustrated in Figures 2 and 3a, the X, Y, control 14 may be operably attached to the base 24. The X, Y controller 14 should be capable of microfine and repeatable movement so that the attached deposition substrate 25 can be precisely positioned in a repeatable manner underneath the deposition probe 12. The operative end of the X, Y controller 14, as illustrated in Figure 2, may be positioned in such a manner that the controller will move the deposition substrate 25 underneath the deposition probe 12 with micron precision and will also be able to move the substrate 25 out of the way to allow the X, Y translation stage 18 to move the loading substrate 27 under the probe 12.

One X, Y controller 14 may be a piezo driven inchworm precision mechanical stage. The inchworm mechanism may have a significant range of motion while maintaining the microfine precision desirable for the present invention. Such a stage may have approximately 20 nm spatial resolution in the X and Y planes and may further utilize encoders to ensure repeatability. The stage may be fitted with a plate designed by those skilled in the art to hold the sample deposition substrate 25. One inch worm stage that may be useful is commercially available from Burleigh Instruments, Burleigh Park, P.O. Box E, Fishers, N.Y. 14453-0755.

In an alternative embodiment, a piezo driven flexure stage may also be utilized as the X, Y controller 14. A piezo driven flexure may have essentially the same precision as the inchworm stage. In still a further embodiment, a linear piezo ratchet mechanism, such as is available from NanoMotion, Israel, may be utilized. Figure 2 illustrates an X, Y controller 14 with a separate motor for the X and Y direction, although various designs may be utilized.

X, Y Translation Stage 18

With reference to Figures 2 and 3b, the translation stage 18 may be further herein described. The X,Y translation stage 18 is operably attached to the base 24 in a position relative to the Z controller 16 and the deposition probe 12 such that it operably interacts with the same. In the present embodiment, the operative end of the X, Y translation stage 18 is fitted with a loading substrate 27 pre-constructed with one or more deposition materials placed thereon. The loading substrate 27 may be operably affixed to the X, Y translation stage 18 in much the same manner as the deposition substrate 25 is attached to the X, Y controller 14. As illustrated in Figures 2 and 3b, the X, Y translation stage may be positioned such that the loading substrate 27 can be moved into an operable position underneath the deposition probe 12.

In one embodiment, the X, Y translation stage 18 may utilize the same type of X, Y positionable inchworm or piezo device as the X, Y controller 14. In alternative embodiments the X, Y translation stage 18 may not require such microfine control since the deposition material may be placed in a much larger, and therefore easily accessible, domain on the loading substrate 27 compound with the domain created on the deposition substrate 25. As illustrated in Figure 2, the present embodiment X, Y translation stage 18 may have much the same design as the X, Y controller 14.

In further embodiments, the X, Y translation stage 18 may have such a range of motion that the loading substrate 27 can be loaded in a first position and then transported into a second

position underneath the deposition probe 12. In this manner, the loading substrate 27 may be cleaned and reloaded with a second deposition material after the first deposition material is loaded onto the probe, all in an automatic fashion.

Z Controller 16

With reference to Figure 2, the Z controller 16 of the present invention will be herein further described. The Z controller 16 may be operably attached to the base 24 where it can operably interact with the X, Y controller 14 and the X, Y translation stage 18. The Z controller 16 may freely move in the vertical (Z) direction. The Z controller 16 of the present invention preferably has an accuracy of 200 nm or less in the Z direction so that the arrayer 10 may be able to accomplish repeatable and consistent deposition domains in a high throughput format. It may also be preferable for the Z controller 16 to have lateral repeatability of one micron or less so that the present invention can create high density arrays with as little as 1 to 2 microns, or less, of space between each spot on the array, i.e., the pitch.

In one embodiment, the Z controller 16 may be commercially available from Newport Corporation, P.O. Box 19607, Irvine, CA 929623-9607, product number TSV 150. In this present embodiment, the Z controller 16 stays relatively stationary in the X, Y direction, allowing the X, Y controller 14 and the X, Y translation stage 18 to move the substrates 25, 27 into position. In alternative embodiments, the Z controller 16 may have X, Y mobility without changing the nature and scope of the present invention.

Deposition Probe 12

As illustrated in Figures 2 (fixed to the end of the Z controller 16, but not visible in Figure 2) and 4 the present invention deposition probe 12 may be further described herein. The deposition probe 12 is preferably 100 to 200 microns long and has a tip 13 of roughly 1-20 microns in height. The radius of curvature of the tip 13 may be approximately 10-50 nm. In one embodiment the probe is modified with a 5-10 micron diameter sphere mounted on the end of the cantilever. The manner in which the sphere can facilitate loading of the probe 12 and deposition of the deposition material may be further described in the above referenced patent application. Furthermore, the operative attachment of such a probe 12 to a Z controller 16 is well known to share in the art and need not be described here.

A commercially available probe may be utilized as the deposition probe 12 of the present invention. Such a probe may be a standard silicon nitride AFM probe available from Digital Instruments/Veeco, 112 Robin Hill Road, Santa Barbara, CA.

Humidity Controller 20

As illustrated in Figures 2 and 5, the humidity controller 20 of the present invention will be herein described. As illustrated in Figure 2, the controller 20 may be operably affixed to the base 24. As illustrated in Figure 5, the humidity controller 20 may further comprise a humidity source 30, a gas flow monitoring and control apparatus 32 (not shown) a gas source 38, a first solenoid valve 40, a second solenoid valve 42, and interconnective tubing 44. The humidity source 30 may be operably positioned to effectively and accurately control the humidity around the deposition probe 12 during the loading and deposition of the deposition material. The monitoring system 32 may be positioned between the humidity source 30 and the deposition probe 12 and controlled by the computer 22. The gas source 38 may be operably connected to the first solenoid 40 and the humidity source 30 by the tubing 44. The gas source may be further connected to the second solenoid 42 by tubing 44 bypassing the humidity source. Furthermore, as shown in Figure 2, tubing 44 may channel the gas to the probe 12. The humidity controller 20 of the present invention may allow for the reproducible deposition of samples in sub-micron and nanometer domains.

The humidity source 30 of the present embodiment utilizes a wetted piece of filter paper or a sponge in a plastic cartridge. A dry inert gas, such as argon, is placed into the cartridge from the gas source 38 and kept under a positive pressure though the use of the solenoid valve 40 controlled by the control system. As illustrated in Figure 5, the gas is discharged by the humidity controller 20, through the solenoid valve 40 and the humidity source 30, past the monitoring and control approaches 32 to flow over the deposition probe 12 and to increase the relative humidity around the probe 12 in such a manner as to effectuate the loading or deposition of the deposition material.

As illustrated in Figure 5, the second solenoid 42 may also draw gas from the gas source 38, but route the gas through tubing 44 that goes around the plastic cartridge 36 and then to the monitoring and control apparatus 32. In this manner, dry gas may be delivered to the deposition probe 12. The solenoid 42 is controlled by the computer 22 and the monitoring apparatus 32 in such a manner that dry gas is mixed with humid gas to achieve the desired humidity level before

reaching the probe 12. Furthermore, after the deposition material is placed on the deposition probe 12, or the deposition substrate 25, the dry gas solenoid 42 may be used to blast dry gas over the deposition probe to dry the deposition material on the probe 12 or on the deposition substrate 25. As may be appreciated, the output from the solenoids 40, 42 may be routed through the monitoring apparatus 32 attached to the monitoring system 32 so to improve repeatability and optimal deposition conditions for various deposition materials. A numerical value may be assigned to each flow rate; monitoring and variations of this numerical value may aid in achieving the desired humidity levels.

In alternative embodiments, a more sophisticated humidity generator may be utilized so that the present invention can further increase the precision and repeatability of the relative humidity surrounding the sample. In yet another embodiment, the dry air may be continuously blown over the deposition probe 12, briefly stopped during the wet gas blast, and then immediately turned on again to minimize sample diffusion on the surface.

In still another embodiment, a constant, humid environment may be adequate for sample loading and deposition. For this embodiment, the present invention may include a plastic chamber or room that envelopes the deposition probe 12, the operative ends of the X, Y controller 14, and the X, Y translation stage 18, or the entire instrument. The chamber or room may be filled with a gas of the desired humidity for the duration of the loading and deposition program.

Control Computer 22

With reference to Figures 1 and 2, the control computer 22 will be herein described. The control computer may be a standard computer utilizing a Pentium, Athlon, or other computer chip with a standard operating environment that includes a monitor, hard drive, etc. The present embodiment may utilize a standard data acquisition computer board commercially available from National Instruments, 11500 Mopac Expressway, Austin, TX 78759-3504, product number PCI-6025e. Such an acquisition board may compile the necessary data to control the humidity, the height of the deposition probe 12, the relative positions of the Z controller 16, the X, Y controller 14, the X, Y translation stage 18, and may also monitor the positions that the deposition material is placed on the deposition substrate 25. Standard or customized software may be loaded onto the computer 22 and may control the operation of the data acquisition board. Customizable software of particular use may be available from LabView.

In addition to the computer controller 22, a stepper motor controller card (A-100 from Mill-Shaf Technologies, Inc.) may be utilized to control the fine action of the X, Y controller 14, the Z controller 16, and the X, Y translation stage 18. The stepper motor controller card of the present embodiment may also be controlled by the LabView (National Instruments) software or other software written by those skilled in the art.

Force Feed Back Monitor 50

With reference to Figure 2, the force feed back monitor 50 may be further described herein. As previously noted, the force feed back monitor 50 may be operably attached to the Z controller 16 and the control computer 22. The force feed back monitor 50 may be able, along with the control computer 22, to accurately recognize when the deposition probe 12 and the loading substrate 27, or the deposition probe 12 and the deposition substrate 25 touch. Knowing the exact moment of contact between and probe 12 and the substrate 25, 27 may more accurately allow transferal of the deposition material from the loading substrate 27 to the deposition probe 12 and from the deposition probe 12 to the deposition substrate 25. A force feed back monitor 50 coupled with the control computer 22 may be known to those in the art for achieving such a result.

In alternative embodiments, the force feed back monitor 50 may only be used to determine the initial relationship of the substrates 25, 27 and the probe 12.

Utilizing the present invention arrayer 10 the probe 12 may be brought into contact with the substrate 25, 27 and then drawn back up to 1 mm or more before being exposed to the humid gas which causes the capillary bridge to form, thus loading or depositing the deposition material. Once the position of the substrate 25, 27 is determined relative to the probe 12, the computer 22 may simply bring the probe 12 to the desired level above the substrate 25, 27 for the subsequent depositions without having to touch the surface of the substrate 25, 27.

Various types of force feed back monitors 50 useful for the above may be known to those skilled in the art.

One commercially available force feed back monitor may be an AFM head from a Dimension 3100 series scanning probe microscope available from Digital Instruments. Other force feed back monitors may be utilized by those of reasonable skill in the art without changing the nature and scope of the present invention. In the present embodiment, the read-out of the monitor 50 may be read through a standard break-out box and fed directly into LabView. In

operation, a deflection value may be established as the threshold value at which LabView will stop the Z controller 14. Thus, once the surface is "found," the instrument of the present invention may be programmed to move the Z controller 14 to within 200nm of the same position repeatedly. In this manner, the instrument may approach and retract from the surface rapidly without the necessity of slowing and carefully counting steps until contact is made on each deposition cycle.

Optical Microscope 52

With reference to Figure 2, the optical microscope 52 may be further herein described. As illustrated in Figure 2, the optical microscope 52 is mounted underneath the optical plate in an inverted position. The optical microscope 52 allows the user to visualize the loading and deposition steps from below the deposition probe 12. Such monitoring may be within the resolution limits of the far field optics of a standard microscope that includes 10X, 20x, 40x, and 60x magnification options with a 10x eyepiece. In still further embodiments, such a microscope may be fitted with a camera for image output to the computer 22, to a separate monitor or to a recording device. As may be appreciated by those skilled in the art, the microscope may be excluded from the present invention arrayer 10 without changing the nature and scope of the invention.

Although the deposition domains may be smaller than the wavelength of the light being used, they are separated by distances on the order of 2 microns, allowing them to be separately observed by virtue of their optical characteristics. This is analogous to far field optical observation of sub-wavelength objects such as individual DNA molecules and manometer scale colloidal metals by virtue of light collected from intercalated fluorophores or reflected photons, respectively. This, optical monitoring may be a useful method for preliminary evaluation of the deposition event as performed by the present invention.

Method of Use

The method of use of the present embodiment will now be herein described. The Z controller 16 is used to bring the probe 12 into contact, or near contact, with the loading substrate 27. Contact force is regulated by monitoring the cantilever deflection signal in LabView through the force feed back monitor 50. A blast of humid gas is then utilized to create a capillary bridge between the probe 12 and the loading substrate 27. This capillary bridge transfers some amount

of the deposition material to the probe 12. The deposition probe 12 is then withdrawn using the Z controller 16. The loading substrate 27 is then moved by the X, Y translation stage 18 out of position beneath the probe 12. The X, Y controller 14 then moves the deposition substrate 25 into position underneath the probe 12. The probe 12 is then brought down into position by the Z controller 12 and the humidity cycle repeated to deposit the deposition material on the deposition substrate 25.

As may be appreciated, this process may be carried out many times before the deposition probe 12 is significantly depleted of deposition material. Thus, one to several deposition domains for each array can be constructed after loading the probe 12 just one time. Each time a new deposition material is deposited, the deposition probe 12 is cleaned. In one embodiment, the probe 12 may be cleaned with UV or ozone burst before loading a second deposition material.

In one embodiment, a sample of protein at a concentration of about 0.1 mg/ml in PBS (a buffered saline solution) may be deposited as a microdrop on a clean glass surface and dried to serve as the deposition materials/loading substrate. The deposition tool may be allowed to contact the dried microdrop and the humidity controlled to allow adsorption of protein to the deposition probe tip 13. This process typically results in loading of the deposition tool with sufficient material for 10 to 100 deposition events. The loaded deposition probe 12 is then utilized to deposit the PBS onto a freshly prepared gold or gold/alkanethiolate surface.

Each cycle of loading the probe and making one domain on the deposition substrate may take as little as 1 minute. In addition, the actual deposition event is relatively short, so the difference between making one and several spots with a single source material is only a few seconds at most. Thus, to build one, or many 10 x 10 molecular arrays of 100 <u>different</u> molecular species may take approximately 1 hour and 40 minutes. In alternative embodiments, this process may be further streamlined and scaled up to allow construction of much more complex arrays (hundreds to thousands of molecular species), and larger numbers of arrays in a similar time frame, without changing the nature and scope of the present invention. All of these steps may be coordinated through LabView utilizing the computer 22.

In still further embodiments, there may be several X, Y translation stages 18 to bring loading substrates 27 into an operable position underneath the deposition probe 12. In this manner multiple deposition materials can be accessed on the multiple loading substrates 27, allowing for the creation of an extremely diverse array.

In yet another alternative embodiment, the optical microscope 52 may be utilized to locate registration marks for sample deposition in defined physical locations.

In another embodiment, the probe may be washed using a microfabricated well with a simple fluidic feed. The washing solution (e.g., water) may be fed into the device, forming a protruding bubble held in place by surface tension. The deposition tool may then be washed in the bubble by piezo driven oscillation of the bubble in the probeiz.

As will be appreciated by those skilled in the art, spot size will be a function of the radius of curvature of the deposition tool, tool and surface hydrophobicity/hydrophilicity, and the control of humidity during the deposition event. The present invention may allow spot sizes in the 200 nm diameter range (tool radius is typically 40 nm) reproducibly when the appropriate parameters are carefully monitored. It is noteworthy that spots quite a bit smaller than this may be possible depending on the sample material and the purposes envisioned for the deposition domain.

The information and examples described herein are for illustrative purposes and are not meant to exclude any derivations or alternative methods that are within the conceptual context of the invention. It is contemplated that various deviations can be made to this embodiment without deviating from the scope of the present invention. Accordingly, it is intended that the scope of the present invention be dictated by the appended claims rather than by the foregoing description of this embodiment.